Effects of Liquid Phase Composition on Salt Cluster Formation in Positive Ion Mode Electrospray Mass Spectrometry: Implications for Clustering Mechanism in Electrospray

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Potassium bromate salt clusters, $[KBrO_3]_nK_x^{x+}$, formed by electrospray ionization were studied as a function of solution properties. Clusters with up to 4 positive charges were observed. Their abundance, charge state and distribution were shown to vary with the organic solvent in solution. The effects of 7 solvents, including methanol, ethanol, isopropanol, acetonitrile, acetone, pyridine, and 1,4-dioxane, were thoroughly investigated. Solvents with a low dielectric constant and a high viscosity seem to favor clustering in solution but do not systematically allow high charge state ion formation. On the other hand, cluster charge reduction during desolvation was not correlated with solvent cation affinity over the range of solvents examined. However, ion distribution in mass spectra could be rationalized as a combination of these two competing phenomena. Charge state increases with the cluster size but may be reduced during ion desolvation when high cation affinity solvent molecules are actually involved in the ion solvation shell. This assumption could be envisaged in either Iribarne or Dole mechanisms of ion release in the gas phase. However, intensity profiles of multiply charged clusters could only be understood in terms of the ion evaporation mechanism. (J Am Soc Mass Spectrom 2001, 12, 1077–1084) © 2001 American Society for Mass Spectrometry

From the beginning of electrospray (ES) mass spectrometry, it has been clear that the yield, abundance and charge state of ES ions depend very strongly on the composition of the solution from which they are formed [1–3]. Molecular ion response for many compounds was shown to increase with an increasing proportion of organic solvent as spray performance improves with a solution of lower surface tension [4]. However, during the course of the electrospray ionization (ESI) process, the solution properties are expected to be altered. Electrochemical reactions at the tip of the capillary [5], droplet formation and solvent evaporation contribute to changes in the analyte concentration and the composition of the sprayed solution.

To gain further insights into the ESI process, salt cluster ions have been utilized [6, 7]. Original salt concentration [1, 8–13], solution conductivity [11]and viscosity [14], and proportion of organic solvent [15, 16]have been shown to influence salt clustering. Solvent polarity [10, 17], nature of the counterion [16, 17], electrolytes present in solution [17]were found to affect the distribution of salt cluster ions in the mass spectrum. In their pioneering work on the effect of various physical and chemical variables on ES production of cluster ions, Meng and Fenn [10]argued that most of their mass spectrometric observations could be explained in terms of the ion evaporation model (IEM) developed by Iribarne and Thomson [18, 19]. The IEM assumes a sequence of solvent evaporation/coulomb explosions to produce very small charged droplets from which solute ions can be desorbed in the ambient gas, and predicts the dependence of ion evaporation rate on ion solvation energy. In contrast, in most subsequent ESI cluster studies [1, 6, 9, 12, 13, 20, 21], experimental results were rationalized by an extended charged residue model (CRM). The original CRM, proposed by Dole [22], holds that the repeated sequence of solvent evaporation/Rayleigh instabilities produces ultimate drop-

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Table 1. Composition of the mobile phase in each solvent experiment

	Methanol (MeOH)			Ethanol (EtOH)			Isopropanol (IpOH)			Acetonitrile (AcN)			Acetone (AcO)			1,4-dioxane (Diox)			Pyridine (Pyr)		
	F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3
Volumic fraction	0.20	0.40	0.70	0.25	0.50	0.80	0.30	0.60	0.80	0.25	0.50	0.75	0.30	0.60	0.80	0.35	0.60	0.80	0.35	0.60	0.80
Molar fraction	0.10	0.23	0.51	0.09	0.23	0.55	0.09	0.23	0.48	0.10	0.25	0.50	0.10	0.27	0.50	0.10	0.24	0.46	0.11	0.25	0.47

lets which contain only one molecule of solute. The molecule becomes a gas phase ion as the last solvent molecule is evaporated from the charged droplet. As a result, the maximum charge that the ion can carry is the final droplet charge. The extended CRM [7] is described as a combination of both mechanisms acting in concert: only singly-charged clusters would be generated by the IEM and the associated charge depletion on the droplet surface would determine the final droplet charge in the CRM.

Inorganic cluster studies with ES have been performed mainly with alkali halide salts. However, while developing a coupling system between ion chromatography and mass spectrometry for the analysis of bromate ions in water [23], strong clustering effects were observed with alkali oxyhalide molecules. Results from experiments conducted with several solvents added to aqueous potassium bromate solutions are reported here and the influence of liquid phase properties on positive ES mass spectrum of inorganic salt clusters is presented.

Experimental

All experiments were performed with a Sciex API III Plus triple quadrupole system with a pneumatically-assisted electrospray interface (Sciex, Thornhill, Canada). The interface temperature was 54 °C throughout all experiments. Ultrahigh-purity nitrogen (UHP, 99.999%) was used as curtain gas (flow rate 0.6 L min^{-1}) and zero-grade air was the nebulizing gas (flow rate 0.8 L min^{-1}). Average of 100 consecutive scans were done on each acquisition range (200 Th). Electrospray ionization (ESI) was operated in the positive mode only. The spray voltage was set at 5000 V and the orifice voltage at 50 V throughout all experiments. A Perkin Elmer Series 200 binary pump (Perkin Elmer, Norwalk, CT) was used for mobile phase delivery, at a flow rate of 1 mL min^{-1}, and a split ratio of 1:20 allowed a 50 μL min^{-1} flow rate to the ESI source.

To validate comparisons between multiple mass spectra, the repeatability of instrumental conditions was checked by plotting results from a quality control solution on a control chart.

All organic solvents were HPLC grade solvents from Merck (Darmstadt, Germany). Water was obtained from a Milli-Q water purification system (Millipore, El Paso, TX). Potassium bromate ($KBrO_3$) was from Alfa (Karlsruhe, Germany). Potassium bromate concentration in the solutions was 3×10^{-3} M. Solutions were prepared with water at 3 different organic solvent proportions, as indicated by the molar fractions F1, F2 and F3 in Table 1.

Results and Discussion

From a potassium bromate salt solution in a water/methanol solution (methanol molar fraction F1 in Table 1), ES mass spectrum shows four series of peaks, the most abundant arising from singly-charged clusters, $[KBrO_3]_nK^+$, (S_n in Figure 1), with n ranging from 1 to 14. As generally observed in cluster studies [24–26], the singly-charged ion intensity drops off exponentially as the degree of aggregation, n, increases. Further series contain multiply charged cluster distributions with up to 4 charges. Because of the coincidence in *m/z* values, $[KBrO_3]_{2n}K_2^{2+}$, $[KBrO_3]_{3n}K_3^{3+}$, and $[KBrO_3]_{4n}K_4^{4+}$ overlap with the much more abundant $[KBrO_3]_nK^+$ ion. Therefore ranges of the multiply charged clusters can only be estimated. Doubly-charged ions, D_n, are observed to range from n = 11 to 27, but both the abundance of $[KBrO_3]_{27}K_2^{2+}$ and the isotope distribution of $[KBrO_3]_{14}K^+$ in Figure 1 suggest the presence of D_{28}. Triply-charged clusters, T_n, with n = 28 to 41 are identified in the ES mass spectrum. The existence of $[KBrO_3]_{42}K_3^{3+}$ was indirectly shown in MS/MS experiments where Tr_{41} was identified at *m/z* 2318 in the CID spectrum of *m/z* 2377. Finally, the fourth charge state is observed for clusters with n = 49 to 55. Multiply-charged clusters of nonvolatile species obtained in positive ESI have been described elsewhere in the literature. Arginine cluster ions have been reported with up to four charges [10, 15]. Work by Gamero et al. [27] has shown considerably higher charge states but with a much larger cation and without the benefit of identification through mass spectrometry. Mostly doubly charged ions were observed from inorganic salts [28] until a recent study by Hao et al. [16] in which alkali metal chloride were shown to cluster with up to 6 positive charges. However, high charge state clusters ($x > 2$) were detected under low cone voltage (<30V) conditions only. The current experiments were run with normal cone voltage (50V) and multiply-charged potassium bromate clusters were shown to be sufficiently stable to survive such in-source CID effects.

An important property of multiply charged clusters is their critical size [29], which is the minimal number of salt units required for a cluster to accommodate an additional charge. Interestingly, n = 11 was found to be the critical size for doubly-charged clusters of potassium bromate, as was reported for both NaCl clusters

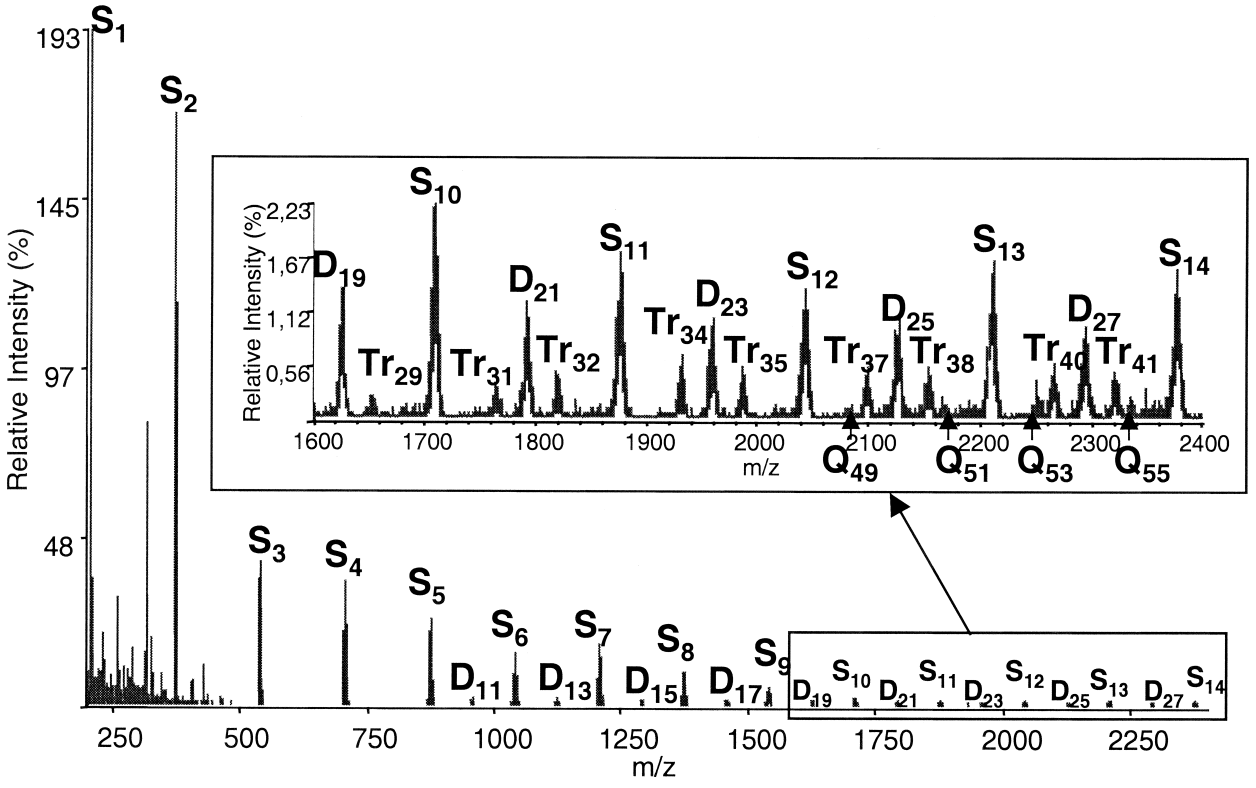

Figure 1. Mass spectrum derived from positive electrospray ionization of potassium bromate in water-methanol matrix (10% molar methanol). S_n designates singly charged $[KBrO_3]_nK^+$ clusters while D_n, Tr_n and Q_n stand for doubly $[KBrO_3]_nK_2^{2+}$, triply $[KBrO_3]_nK_3^{3+}$, and quadruply $[KBrO_3]_nK_4^{4+}$ charged clusters, respectively.

studied by Hao et al. [16] at low cone voltage and by Cooks et al. [28]. Similar minimal n values were observed for $[KBrO_3]_nK_3^{3+}$ (n = 28) and $[NaCl]_nNa_3^{3+}$ (n = 27) [16] from methanol solutions. Cation radius influences the abundances of cationized salt clusters by affecting their solvation energy [25] but Hao et al. [16] did not observe any dramatic effect of the anion radius on the distribution of cluster ions generated in positive ESI. The similar critical size obtained for various salt clusters suggests that electrostatic repulsion forces between charges in a multiply charged cluster is not the main factor that determines ion stability.

Cluster ion abundance is clearly seen to decrease as charge state increases (Figure 1), thus suggesting that the ESI process does not produce as many multiply charged ions as singly charged ones. These relative intensities can also be influenced by quadrupole transmission bias and by in-source CID of large ions. Cluster formation in ESI is primarily due to a concentration effect, as has been widely reported [1, 8–13]. In the present study, a 3 mM potassium bromate solution in water/methanol gave rise to the optimal cluster distribution observed in Figure 1. Maintaining this concentration, abundance and distribution of the cluster ions were shown to be highly dependent on the nature of the organic solvent in solution. This effect is particularly sensitive for the large clusters. Quadruply-charged clusters are absent in mass spectra obtained from an acetonitrile solution and the largest observed clusters contain 3 charges (n = 34 to 41). A restricted range of triply charged clusters is also obtained with slightly higher intensity from acetone solution, together with the largest $[KBrO_3]_nK_4^{4+}$ ions (n = 53 to 55). Moreover, at low organic solvent content in solution, clusters with low abundance are produced from acetone and acetonitrile, as compared to methanol. In contrast, ES of alcohol solutions allows high abundance ions, and at low organic solvent proportion, increasing ion intensities are observed in the order methanol-ethanol-isopropanol. Moreover, additional quadruply (n = 45 and 47) and triply (n = 25 and 26) charged ions appear in mass spectra from ethanol and isopropanol solutions. A potassium bromate solution can well be considered as a mixture of neutral $(KBrO_3)_n$ aggregates, the size of which decreases as the ability of the liquid phase to dissociate the salt increases. Such a trend would be further reinforced as the salt concentration increases in the droplet as the solvent evaporates. Solvents with high dielectric constant are able to increase the degree of solution phase charge separation. The efficiency of charge separation is further controlled by solute ion mobility, which is known to be governed by solution viscosity [14]. Therefore, solutions of acetone or acetonitrile which show both a high dielectric constant and a low viscosity (Table 2) would not favor salt agglomeration, whereas increase of the clustering extent in alco-

Table 2. Effects of organic solvent addition physical properties of an aqueous solution

		MeOH	EtOH	IpOH	AcN	AcO	Diox	Pyr
	F1	73.2	67.4	55.0	73.4	60.1	52.4	51.6
ε	F2	64.6	52.9	40.6	62.6	46.4	27.5	38.1
	F3	50.0	36.0	28.5	48.8	35.5	14.4	22.6
	F1	1475	2170	3015	1113	1500	1681	1698
η (10^5.poises)	F2	1736	2814	3922	983	1456	2266	2495
	F3	1484	2036	3300	648	1075	2058	2224

ε: dielectric constant.
η: viscosity.
Values extrapolated from data in ref. [35].

hol solution can be predicted in the order methanol-ethanol-isopropanol. This result is consistent with data from the literature which showed increasing extent of arginine clustering in the order of decreasing solubility [10]. Potassium bromate cluster abundance in ES mass spectra could then be correlated to aggregation phenomenon in the liquid phase. As reported by one referee, another aspect could be considered. During the ESI process, solvent evaporation from the surface of droplets of liquid salt solutions easily leads to clustering of salts in the surface layer and accordingly solvent evaporation from charged droplets to the formation of salt cluster ions in a surface layer. The formation of such surface layers prevent a fast evaporation of the solvent, allowing a slow electrohydrodynamic process leading to the formation of charged cluster ions. The increase of the cluster size with a decrease of the solubility of salts in the solvent used can then be explained by a precipitation of salt at the surface of the liquid (i.e., at a phase boundary).

However these simplistic models seem mainly to account for the dominant effect of liquid phase properties in the case of low organic solvent proportion. Indeed, as the solution is enriched with organic solvent, relative abundance of cluster ions can no longer be predicted from the clustering capabilities of the liquid phase. The abundance of potassium bromate clusters as a function of the alcohol proportions (Figure 2) largely depends on the nature of the alcohol in solution. While ion intensity increases with methanol content, enrichment of a solution with isopropanol causes an overall decrease of cluster signal. Results from ethanol experiments show an intermediate case where increasing cluster abundances are measured as organic solvent is added until a maximum is reached. It should be noted that the extent of signal decrease is larger for higher charge state clusters. A decrease in signal intensity is also observed in experiments conducted with other solvents. For example, quadruply charged ions disappear from the ES mass spectrum as the liquid phase is enriched with acetone. Such ion signal suppression has already been shown for high organic solvent proportion [4, 30] due to the higher proton affinity of methanol and acetonitrile, as compared to water. Cole et al. [17] showed that solvent polarity contributes heavily to the

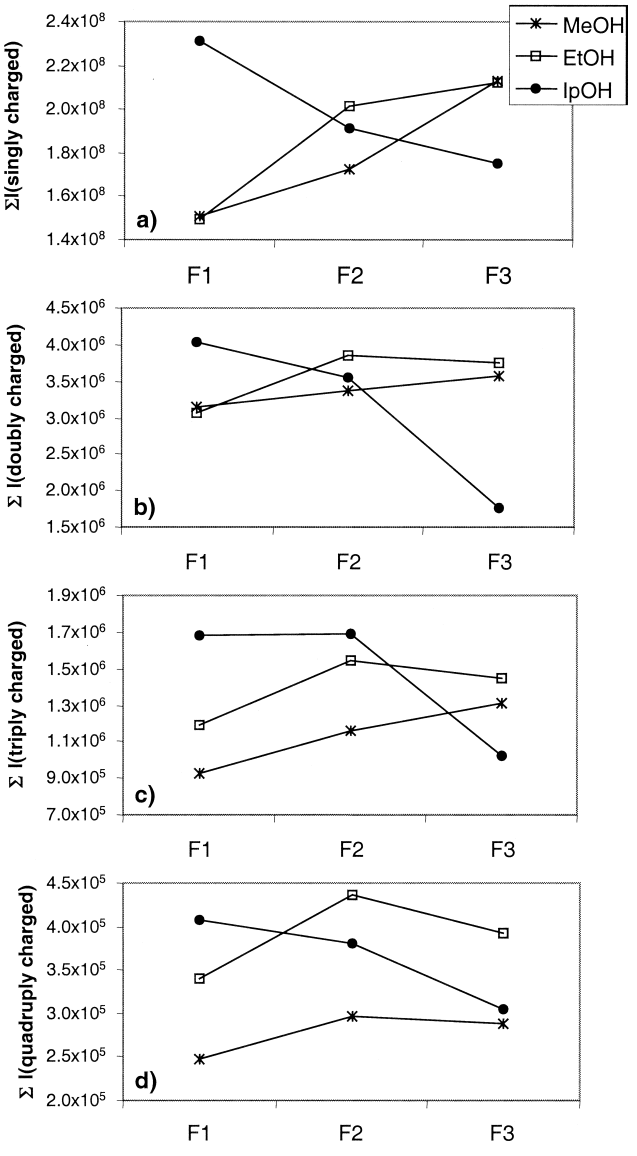

Figure 2. Effect of alcohol solvent content (F1, F2, and F3 defined in Table 1) in solution on the intensity of **a**) singly-, **b**) doubly-, **c**) triply-, and **d**) quadruply-charged potassium bromate clusters. MeOH, EtOH and IpOH stand for methanol, ethanol and isopropanol, respectively.

determination of cluster charge state in negative ES MS. In contrast, Hao et al. [16] reported that, in the positive ion mode, the distribution of salt cluster ions was not dramatically affected by varying the nature of the organic solvent in solution. Potassium bromate clusters clearly do not behave as alkali metal chloride clusters towards the nature of solvent in solution.

Another interesting feature is the fact that cluster ion critical size depends on the nature of the organic solvent (Table 3). These values remain constant throughout the entire solvent concentration range investigated. For a given charge state, the smallest clusters are also expected to be the less stable as repulsive forces between the charges increase as the distance between these charges decreases. The critical size values may reflect

Table 3. Critical size of triply- and quadruply-charged clusters from various solvent solutions

	+3	+4	Proton affinity (kcal/mol)[a]
Ethanol	25	45	185.6
Isopropanol	25	45	189.5
Dioxane	25	45	190.6
Methanol	28	49	180.3
Pyridine	31	49	222.0
Acetone	34	53	194.0
Acetonitrile	34	—	186.2

[a]From [36]. Solvent cation and proton affinity scale are assumed to be directly correlated.

either stabilizing effects or cation affinity of the solvent. The observed clusters are naked ions, so stabilizing effect of the solvent could only be envisaged during the agglomeration process in solution. In other words, the structure and so the stability of the cluster would thus be solvent dependent. This remains highly speculative as no such data have been reported in the literature. On the other hand, relative cation affinity of the solvent and the cluster may determine the final ion charge state during the last desolvation stage, as represented below:

$$\text{Solvent}_m\,[\text{Salt}]_n\,\text{Cat}_z^{z+} \rightarrow [\text{Salt}]_{n-i}\,\text{Cat}_{z-j}^{(z-j)+} + i\,\text{Salt} + j\,\text{SolventCat}^+ + (m-j)\,\text{Solvent}$$

where the cluster of n salt units carrying z positive charges (Cat) solvated with m solvent molecules desolvates under in-source CID conditions. However no correlation can be established between critical size values and solvent cation affinity (Table 3).

Intensity distribution of multiply charged clusters is also solvent dependent, and two different solvent groups can be identified this way. The first group is composed of the alcohols and 1,4-dioxane, and is illustrated by isopropanol results shown in Figure 3. Its main characteristics are (i) a decreasing profile of doubly-charged ions at low organic solvent content, which becomes nearly constant in solvent enriched solutions, and (ii) an extended range of triply-charged clusters whose intensities are observed to increase with size. The second defined solvent group (acetonitrile, acetone and pyridine) allows only partial observation of the +3 and +4 ions and increasing intensities are measured for the doubly charged clusters as n increases (Figure 3). Cluster charge state reduction during desolvation could explain the absence of the smallest triply charged clusters and the subsequent enhancement of the largest doubly charged ions in mass spectra obtained at high solvent concentration in the second group. For many practical reasons, ESI MS is often carried out with solvents comprising mixtures of two or more components which have different vapor pressures. Solvent fractionation is most likely to occur during droplet evaporation and it was shown to be correlated to relative vapor pressures of the solvents [31]. A solvent with a low vapor pressure would likely slow droplet evaporation leading to poor ionization efficiency. Although acetonitrile is more volatile than isopropanol (Table 4), lower ion abundances are measured from acetonitrile solutions compared to isopropanol. The influence of solvent volatility was not observed in these experiments. This result is consistent with the fact that pneumatically assisted nebulization reduces the spray dispersion, leading to an increase of local vapor pressure and so to a slowing of solvent fractionation [31]. To understand the observed solvent dependence of cluster intensity profiles, parameters involved in the release of cluster ions in the gas phase have been more closely scrutinized.

The ion-evaporation-controlled charged-residue mechanism predicts that the ranges of cluster size, n, for charge state z and z + 1 are expected to be contiguous without either overlapping or leaving empty spaces in between [7]. In this work, the bands in which the various charge states of potassium bromate clusters appear follow each other but overlap substantially (n = 0–14 for z = 1; n = 11–28 for z = 2; n = 25–42 for z = 3; n = 45–55 for z = 4, from isopropanol solutions). Many clusters are shown to accommodate two different charge states, and the band overlap might even be larger than actually observed within our limited *m/z* range. Moreover, if gas phase cluster ions arise from desolvated charged residue, the increase of cluster critical size would be expected to become larger as the

Table 4. Physico- and thermo-chemical properties of pure solvents at 20 °C (otherwise indicated)

	Water	MeOH	EtOH	IpOH	AcN	AcO	Diox	Pyr
ε[a]	80.4	33.0	25.5	18.6	36.1	20.1	1.6	12.3
σ[a] (dynes/cm)	72.7	22.6	22.7	21.7	28.4	22.7	35.4	36.4
η[a] (10^5.poises)	1004	599	1154	2470	383	327	1281	1058
Vapor pressure[b] (Torr) at 25 °C	24	125	59	42	90	203	42	18
Proton affinity[c] (kcal/mol)	165.0	180.3	185.6	189.5	186.2	194.0	190.6	222.0
π^*[d] (Kamlet and Taft's index)	1.09	0.60	0.54	0.48	0.66	0.62	0.49	0.87
$\Delta\pi^*$ ($\pi^*_{\text{water}}/\pi^*_{\text{organic solvent}}$)	—	1.82	2.02	2.08	1.65	1.76	2.22	1.25

[a]from [35].
[b]extrapolated from data in [37].
[c]from [36].
[d]from [38].

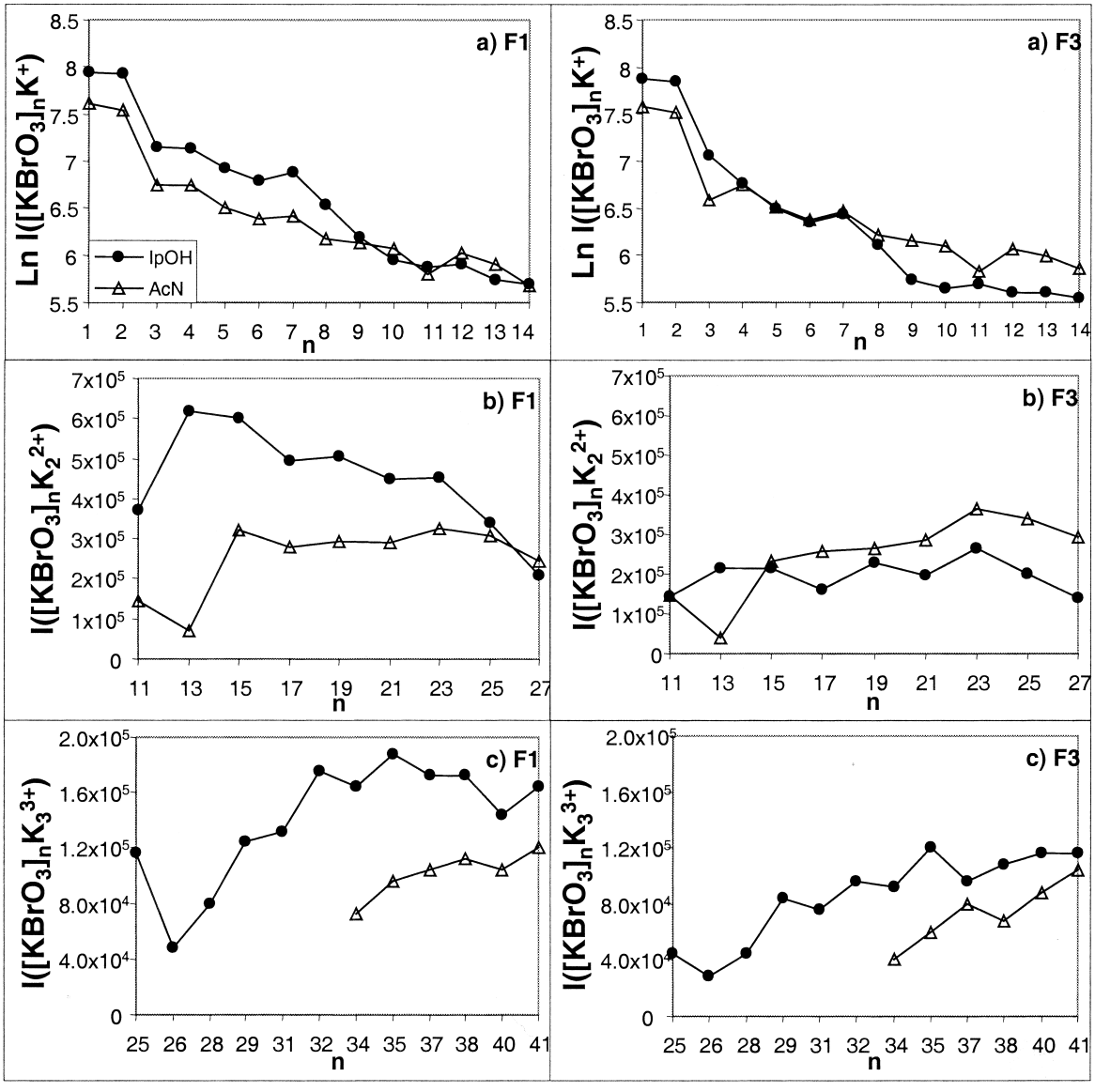

Figure 3. Intensity distribution of **a**) singly-, **b**) doubly-, and **c**) triply- charged potassium bromate clusters from isopropanol (IpOH) and acetonitrile (AcN) solutions at two different solvent compositions (F1 and F3, defined in Table 1).

solvent cation affinity increases. As previously mentioned, no such correlation could be established (Table 3). As far as charge reduction is assumed to occur during ion desolvation, a valuable factor should be the actual involvement of organic solvent molecules in the solvation shell of the cluster ions.

Studies on selective ion solvation in binary solvents [32, 33] generally showed that the ionized solute exhibits a very high degree of preferential solvation by the more polar species. As the two solvent species become more dissimilar in terms of dipolar properties, the observed degree of preferential solvation for the ionized solute was shown to be correspondingly more prominent [32]. Of the many empirical parameters which have been proposed, the Kamlet and Taft's index (π^*) [34] measures a specified combination of polarity and polarizability. The degree of preferential solvation by organic solvent in each binary mixture should then vary according to the value of as $\Delta\pi^*$ (Table 4), defined as $\Delta\pi^* = \pi^*(\text{water})/\pi^*(\text{organic solvent})$. The higher $\Delta\pi^*$ value, the larger the amount of preferential ion solvation by water molecules. Accordingly, involvement of organic solvent molecules in ion solvation shell is expected to be higher from acetonitrile solutions, as compared to isopropanol solutions. Despite the fact that acetonitrile exhibits a lower cation affinity than isopropanol, a larger charge reduction effect can then be expected when spraying acetonitrile solutions.

The charge state dependence of multiply-charged ion intensity profiles (Figure 3) has also been studied. While the low intensity of the smallest triply-charged clusters can be attributed to their limited stability, it

contrasts with the decreasing trend exhibited by $[KBrO_3]_nK_2^{2+}$ ions as n increases, particularly at low solvent proportion in solution. These results can well be understood with the IEM, particularly by the derived model presented by Fenn [2]. This model postulates that, although the interaction with two surface cations would be easier for the largest salt agglomerates, a high charge density should be reached at the droplet surface so that such ions can be emitted in the gas phase according to a field emission process. The decreasing trend observed for doubly-charged ion intensity as n increases would indicate that, for this required charge density, small agglomerates (such as n = 11) would readily interact with two charges. As the free enthalpy of transfer of an ion from a small charged droplet to the gas phase increases with the ion size, it follows that the smallest doubly-charged clusters would desorb at a rate faster than the larger ones. The opposite trend is observed for triply-charged clusters, thus suggesting that the threshold field required to evaporate the largest ions is reached before the smallest agglomerates are able to interact with three surface charges.

This model contrasts with Gamero et al. interpretation that only singly-charged clusters can be produced by the IEM [27]. However, this conclusion was drawn from experiments using highly conductive solutions and large salt agglomerates are not expected to form readily in such conditions. Hence, solvent evaporation leading to the formation of a charged residue would likely be the formation process of the large multiply-charged clusters observed in Gamero's experiments, where droplet solvent evaporation would occur at a faster rate than salt agglomeration does. In contrast, salt agglomeration can occur to a great extent in less conductive solutions such as the potassium bromate solutions used in this study. Neutral salt agglomerates could then form in the droplets and interact with surface charges before all the solvent molecules are evaporated, possibly resulting in charged cluster ion desorption into the gas phase according to the IEM mechanism.

Conclusion

Potassium bromate salt clusters with various charge states were determined to offer advantages to study the role of organic solvent in the ESI mechanism. Relative abundance, charge state, and intensity distribution of these clusters were shown to depend on both the nature and the amount of organic solvent in solution. In the liquid phase, the extent of salt clustering is seen to be controlled solely by solution dielectric constant and viscosity. Results obtained from multiply-charged clusters (mainly +2 and +3 ions) are rationalized with the ion evaporation mechanism. Particularly, the charge state dependence of ion intensity profiles can be understood by the IEM-derived model proposed by Fenn [2]. Selective solvation of inorganic ions in binary mixtures is required to account for the solvent dependence of ion distributions. It follows that the extent of cluster ion charge reduction during desolvation can be correlated quite well to the cation affinity of the organic solvent in solution as far as these solvent molecules are largely involved in the ion solvation shell.

References

1. Kebarle, P.; Tang, L. From ions in solution to ions in the gas-phase: The mechanism of electrospray mass-spectrometry. *Anal. Chem.* **1993,** *65,* 972A–986A.
2. Fenn, J. B. Ion formation from charged droplets: Roles of geometry, energy, and time. *J. Am. Soc. Mass Spectrom.* **1993,** *4,* 524–535.
3. Wang, G. D.; Cole, R. B. Mechanistic interpretation of the dependence of charge-state distributions on analyte concentrations in electrospray-ionization mass-spectrometry. *Anal. Chem.* **1995,** *67,* 2892–2900.
4. Zhou, S. L.; Hamburger, M. Effects of solvent composition on molecular ion response in electrospray mass spectrometry: Investigation of the ionization processes. *Rapid Commun. Mass Spectrom.* **1995,** *9,* 1516–1521.
5. Blades, A. T.; Ikonomou, M. G.; Kebarle, P. Mechanism of electrospray mass-spectrometry: Electrospray as an electrolysis cell. *Anal. Chem.* **1991,** *63,* 2109–2114.
6. Fernandez de la Mora, J. Electrospray ionization of large multiply-charged species proceeds via Dole's charged residue mechanism. *Anal. Chim. Acta* **2000,** *406,* 93–104.
7. Gamero-Castano, M.; Fernandez de la Mora, J. Mechanisms of electrospray ionization of singly and multiply charged salt clusters. *Anal. Chim. Acta* **2000,** *406,* 67–91.
8. Ikonomou, M. G.; Blades, A. T.; Kebarle, P. Investigations of the electrospray interface for liquid-chromatography mass-spectrometry. *Anal. Chem.* **1990,** *62,* 957–967.
9. Ikonomou, M. G.; Blades, A. T.; Kebarle, P. Electrospray ion spray: A comparison of mechanisms and performance. *Anal. Chem.* **1991,** *63,* 1989–1998.
10. Meng, C. K.; Fenn, J. B. Formation of charged clusters during electrospray ionization of organic solute species. *Org. Mass Spectrom.* **1991,** *26,* 542–549.
11. Tang, L.; Kebarle, P. Effect of the conductivity of the electrosprayed solution on the electrospray current: Factors determining analyte sensitivity in electrospray mass-spectrometry. *Anal. Chem.* **1991,** *63,* 2709–2715.
12. Tang, L.; Kebarle, P. Dependence of ion intensity in electrospray mass-spectrometry on the concentration of the analytes in the electrosprayed solution. *Anal. Chem.* **1993,** *65,* 3654–3668.
13. Zhou, S. L.; Hamburger, M. Formation of sodium cluster ions in electrospray mass spectrometry. *Rapid Commun. Mass Spectrom.* **1996,** *10,* 797–800.
14. Zhou, S. L.; Cook, K. D. A mechanistic study of electrospray mass spectrometry: Charge gradients within electrospray droplets and their influence on ion response. *J. Am. Soc. Mass Spectrom.* **2001,** *12,* 206–214.
15. Zhang, D.; Wu, L.; Koch, K. J.; Cooks, R. G. Arginine clusters generated by electrospray ionization and identified by tandem mass spectrometry. *Eur. Mass Spectrom.* **1999,** *5,* 353–361.
16. Hao, C.; March, R. E.; Croley, T. R.; Smith, J. C.; Rafferty, S. P. Electrospray ionization tandem mass spectrometric study of salt cluster ions. Part 1: Investigations of alkali metal chloride and sodium salt cluster ions. *J. Mass Spectrom.* **2001,** *36,* 79–96.
17. Cole, R. B.; Harrata, A. K. Solvent effect on analyte charge-state, signal intensity, and stability in negative-ion electrospray mass-spectrometry: Implications for the mechanism of negative-ion formation. *J. Am. Soc. Mass Spectrom.* **1993,** *4,* 546–556.

18. Iribarne, J. V.; Thomson, B. A. On the evaporation of small ions from charged droplets. *J. Chem. Phys.* **1976,** *64,* 2287–2294.
19. Thomson, B. A.; Iribarne, J. V. Field-induced ion evaporation from liquid surfaces at atmospheric-pressure. *J. Chem. Phys.* **1979,** *71,* 4451–4463.
20. Wang, G.; Cole, R. B. Charged residue versus ion evaporation for formation of alkali metal halide cluster ions in ESI. *Anal. Chim. Acta* **2000,** *406,* 53–65.
21. Gamero-Castano, M.; Fernandez de la Mora, J. F. Modulations in the abundance of salt clusters in electrosprays. *Anal. Chem.* **2000,** *72,* 1426–1429.
22. Dole, M.; Mack, L. L.; Hines, R. L.; Mobley, R. C.; Ferguson, L. D.; Alice, M. B. Molecular beams of macroions. *J. Chem. Phys.* **1968,** *49,* 2240–2249.
23. Charles, L.; Pepin, D.; Casetta, B. Electrospray ion chromatography tandem mass spectrometry of bromate at sub-ppb levels in water. *Anal. Chem.* **1996,** *68,* 2254–2558.
24. Campana, J. E.; Green, B. N. Unicluster dissociation of large alkali iodide cluster ions. *J. Am. Chem. Soc.* **1984,** *106,* 531–535.
25. Wang, G. D.; Cole, R. B. Solvation energy and gas-phase stability influences on alkali metal cluster ion formation in electrospray ionization mass spectrometry. *Anal. Chem.* **1998,** *70,* 873–881.
26. Anacleto, J. F.; Pleasance, S.; Boyd, R. K. Calibration of ion spray mass-spectra using cluster ions. *Org. Mass Spectrom.* **1992,** *27,* 660–666.
27. Gamero-Castano, M.; Fernandez de la Mora, J. Kinetics of small ion evaporation from the charge and mass distribution of multiply charged clusters in electrosprays. *J. Mass Spectrom.* **2000,** *35,* 790–803.
28. Zhang, D. X.; Cooks, R. G. Doubly charged cluster ions (NaCl)(m)(Na)(2).(2+): magic numbers, dissociation, and structure. *Int. J. Mass Spectrom.* **2000,** *195/196,* 667–684.
29. Guo, B. C.; Kerns, K. P.; Castleman, A. W. TI8C12+-Metallo-carbohedrened: A new class of molecular clusters. *Science* **1992,** *255,* 1411–1413.
30. Schneider, R. P.; Lynch, M. J.; Ericson, J. F.; Fouda, H. G. Electrospray ionization mass-spectrometry of semduramicin and other polyether ionophores. *Anal. Chem.* **1991,** *63,* 1789–1794.
31. Zhou, S. L.; Cook, K. D. Probing solvent fractionation in electrospray droplets with laser-induced fluorescence of a solvatochromic dye. *Anal. Chem.* **2000,** *72,* 963–969.
32. Day, T. J. F.; Patey, G. N. Ion solvation dynamics in binary mixtures. *J. Chem. Phys.* **1997,** *106,* 2782–2791.
33. Day, T. J. F.; Patey, G. N. Ion solvation dynamics in water-methanol and water-dimethylsulfoxide mixtures. *J. Chem. Phys.* **1999,** *110,* 10937–10944.
34. Kamlet, M. J.; Abboud, J. L.; Taft, R. W. Solvatochromic comparison method .6. Pi-star scale of solvent polarities. *J. Am. Chem. Soc.* **1977,** *99,* 6027–6038.
35. Timmermans, J. *Physicochemical Constants of Binary Systems.* Interscience Publishers Inc.: NY, 1960.
36. Hunter, E. P.; Lias, S. G. *J. Phys. Chem. Ref. Data* **1998,** *27,* 413.
37. *Handbook of Chemistry and Physics*, 71st edition; CRC Press: Boca Raton, FL, 1990.
38. Marcus, Y. *The Properties of Solvents.* John Wiley & Sons: Chichester, England, 1998.